REMARKS

Claim Amendments

Upon entry of the foregoing amendment, claims 1-2, 4, and 6-19 are pending in the application. Claims 1, 2, 6, 16, and 17 have been amended. Claims 3 and 5 have been cancelled herein without prejudice or disclaimer to the subject matter therein. Applicants respectfully request entry of the above amendment and submit that the above amendment does not constitute new matter. Support for the amendments to the claims may be found throughout the specification and in the claims as originally filed. For example, support for the amended claims may be found in paragraph [015] and claim 5 as originally filed.

Claim Rejections - 35 U.S.C. § 112, First Paragraph

The Office Action maintained the rejection of claims 1-6, 16, and 17 under 35 U.S.C. § 112 ¶ 1 because the specification, while being enabled for a method of proliferating cardiomyocytes *in vitro* by introducing adenoviral vectors expressing a D-type cyclin, CDK4, or CDK6 and a nuclear localization signal, allegedly does not reasonably provide enablement for an *in vitro* or *in vivo* method of proliferating any terminal differentiated cell by introducing any cyclin and any cyclin dependent kinase into the cell.

Applicants respectfully disagree and traverse this rejection.

The Office Action states that although every aspect of the invention does not need to be disclosed in order to be enabled, the disclosure of one cyclin/CDK combination delivered by one type of gene therapy vector into one type of cell does not reasonably provide enablement for all cell types, all cyclin/CDKs and all delivery methods.

Applicants have cancelled claims 3 and 5, and have amended claims 1, 2, 6, 16, and 17 to recite "cardiomyocytes." Therefore, as amended, the claims recite a single cell type (cardiomyocytes), specific CDKs (CDK4 or CDK6), and a specific group of cyclins, D-cyclins. The specification also exemplifies several vectors derived from virus such as adenovirus, adeno associated virus, retrovirus, vaccina virus, chick poxvirus and papovirus such as SV40. *See* paragraph [046]. Applicants also note that claim 6 is specifically directed to the use of an adenovirus vector.

As discussed in the specification, when RB (the target of CDK) is phosphorylated, it losses the function to repress E2F and becomes inactive. The inactivation of RB causes the

abnormal regulation of the cell cycle and leads to the malignant transformation of the cell. *See* paragraph [006].

Applicants have found that D-type cyclins and CDK4 induced by mitogenic stimuli remained in the cytoplasm of cardiomyocytes, and did not enter the cell nucleus, whereby neither phosphorylation of RB nor activation of cyclin E/CDK2 took place. Indeed, Applicants demonstrated that several D-type cyclins (e.g., cyclin D1, D2, and D3) remained in the cytoplasm. *See e.g.*, paragraph [066] and Figure 2. From this observation, Applicants constructed adenovirus vectors with a cyclin D1 gene attached and a nuclear localization signal (NLS), as well as a CDK4 gene respectively, and introduced these adenovirus vectors into cultured cardiomyocytes. These viruses caused not only expression of cyclin D1/CDK4 in the nucleus and RB phosphorylation, but also proliferation of cardiomyocytes. *See e.g.*, paragraph [014] and Examples 3 and 4. Accordingly, as acknowledged by the Office Action, the specification teaches the proliferation of cardiomyocytes *in vitro*. *See* Office Action at pages 2 and 6.

The specification also provides an example of proliferating cardiomyocytes *in vivo*. In Example 5, two kinds of adenoviruses (Ad-D1NLS and Ad-CDK4) were injected into the apical region of rat heart. As a control, an adenovirus comprising the *lacZ* gene was also injected as described above. Four days after injections, the hearts were fixed and sections of tissues were stained with anti-Ki-67 antibodies. In the images of heart sections co-infected with the two vectors (Ad-D1NLS and Ad-CDK4), the Ki-67 nuclear protein, which is expressed in proliferating cells in all phases of the cell cycle, was expressed in cardiomyocytes. In the control containing the *lacZ* gene, however, the expression of the Ki-67 nuclear protein was not observed. These results, "strongly suggest that nuclear import of cyclin D1 and CDK4 could promote cell cycle entry of cardiomyocytes in adult hearts" and that "cardiomyocytes obtained the ability of proliferation by the expression of cyclin D1/CDk4 in the nucleus." *See* Example 5.

The conclusions reached in Example 5 have been acknowledged in the art. For example, in one publication, the authors assert that in the heart, evidence that cyclin D1 may play an important role in cardiomyocyte proliferation stems from, *inter alia*, "coinfection of recombinant adenoviruses expressing a variant of cyclin D1 and CDk4 induces pRb phosphorylation and stimulates re-entry into the cell cycle of cardiomyocytes in culture as well as in adult hearts." *See* Ledda-Columbano et al., "Thyroid Hormone Induces Cyclin D1 Nuclear Translocation and

DNA Synthesis in Adult Rat Cardiomyocytes," *The FASEB Journal* 20:87-94, (2006), at page 87; attached hereto as **Exhibit A**. This article cites to Tamamori-Adachi et al. (2003), cited in Appendix A in Applicants' last response. Accordingly, Applicants submit that the art supports the conclusions reached in the specification and therefore, Applicants have enabled the full scope of the claimed invention.

In view of the foregoing, Applicants respectfully request withdrawal of the enablement rejection under 35 U.S.C. § 112, 1st paragraph.

CONCLUSION

An indication of allowance of all claims is respectfully solicited. Early notification of a favorable consideration is respectfully requested.

Respectfully submitted,

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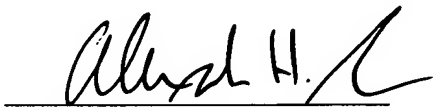
By: 

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